Supplemental data 1: Immunostaining of tumor tissue

Tumor were excised and stored in 10% neutral buffered formalin (Richard Allan Scientific, San Diego, California).

Hematoxilin-Eosin staining

Four-micron sections were cut from formalin-fixed paraffin-embedded (FFPE) tissue blocks, heated at 60°C for 1h and deparaffinized in 3 baths of xylene (HistoPrep, Fisher Scientific, Hampton, New Hamphire). Rehydration was performed by 2 baths of absolute ethanol (Pharmco, Brookfield, Connecticut), followed by 1 bath of 95% ethanol and 1 bath of 70% ethanol. After a wash in distilled water, sections were stained with hematoxylin solution (Gill 3, Thermofisher, Waltham, Massachusetts), followed by one wash in water, incubation for 1 minute in 0.2% ammonia, wash in water and 95% ethanol. Sections were counterstain with eosin solution (Eosin Y Cytoplasmic Counterstain, Thermofisher) and dehydrated with 95% ethanol followed by two baths of absolute ethanol. Slides were washed in xylene three times and mounted in Xylene based mounting media (Richard-Allan Scientific). Stained sections were observed with an Olympus BX51.

CD8 staining

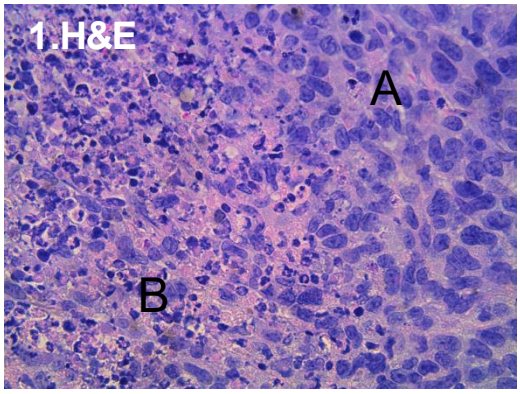
Four-micron sections were cut from FFPE tissue blocks, heated at 60°C for 1h, deparaffinized and rehydrated as described above. Antigen retrieval was achieved by heating the slides in citrate buffer in a pressure cooker with filled in 1L boiled H_2O, microwave at 1000w for 10min. Slides were stained with CD8 antibody (Thermo Fisher, Waltham, Massachusetts) using the DAKO

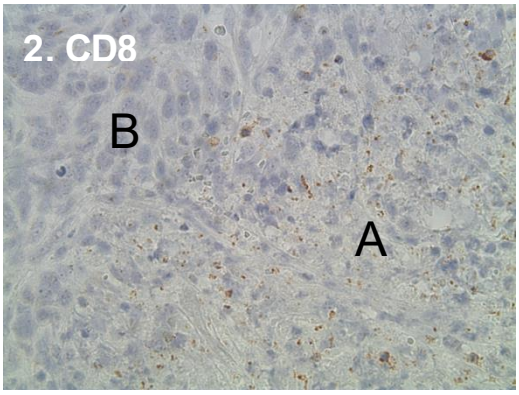
Envision + Dual Link System according to the manufacturer's recommended protocol (Agilent, Santa Clara, CA).

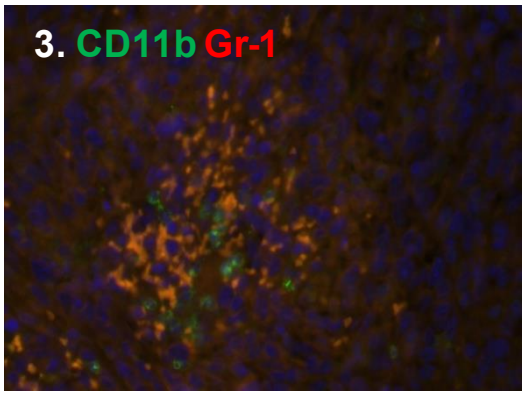
CD11b / Gr-1 staining

Four-micron sections were cut from FFPE tissue blocks, heated at 60°C for 1h, deparaffinized, rehydrated anf heat-treated for antigen retrieval as described above. Superfrost Plus slides (Fisher Scientific, Hampton, New Hampshire) were used. The sections were incubated with 0.1% Triton X-100 for 15min, then with 5% bovine serum albumin for 30min at room temperature. Sections were co-incubated with Anti-Gr1(Biolegend, San Diego CA, 1:2000dilution) and anti-Cd11b (Thermo, Waltham, MA, 1:3000 dilution) for overnight at 4C. After 3 washes in PBS, sections were incubated for 1 h in the dark with FITC-conjugated secondary goat anti-rabbit and chicken anti-rat antibodies (Thermo Fisher). After washing with PBS three times, the tissue sections were covered with ProLong Diamond Antifade Mountant with DAPI (Thermo Fisher). Immunostaining was observed with a conventional fluorescence microscope (Zeiss, Oberkochen, Germany)

The pictures below are representative of the excised tissue in all groups and were taken with a 20X objective. 1: hematoxylin-eosin staining (H&E); 2: CD8 staining; 3: CD11/Gr-1 costaining. A indicates zones with typical organization and cellular morphology of growing, "healthy" 4T1-derived tumor; B indicates portion of the tumor undergoing necrosis and apoptosis, mostly due to lack of blood supply, where CD8+ cells and CD11+/GR-1+ cells are located. The same profiles were observed in the control group and the treatment groups.


1.H&E
A
B


2. CD8
B
A


3. CD11b Gr-1